

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

411 1. (previously presented): A method of screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcription associated biomolecule within a host cell, comprising:

(a) cloning a first nucleic acid fragment that codes for a DNA-binding domain peptide of a transcription activator into a first expression vector to yield a construct (1), wherein said DNA-binding domain peptide binds to a DNA regulatory sequence binding site;

(b) fusing a second nucleic acid fragment into said construct (1), in the same translation reading frame as said first nucleic acid fragment, to yield said first expression vector containing a construct (2) that encodes a chimeric DNA-binding domain/transcription associated biomolecule, wherein said second nucleic acid fragment codes for an antigenic portion of said transcription associated biomolecule that is sufficient to generate antibody capable of binding to said transcription associated biomolecule;

(c) providing said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for said DNA-binding domain peptide;

(d) cloning a third nucleic acid fragment that codes for a single chain antibody into a second expression vector to yield a construct (3), wherein said single chain antibody is expressed in a bio-active form that may bind to said antigenic portion;

(e) fusing a fourth nucleic acid fragment that codes for a trans-activation peptide into said construct (3), in the same translation reading frame as the third nucleic acid fragment to yield said second expression vector containing a construct (4), encoding a chimeric single chain antibody/trans-activation peptide that may bind to said antigenic portion;

(f) introducing said first and second expression vectors into said host, such that both vectors are expressed; and

(g) monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigenic portion within said host cell upon detection of said expression.

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2. (previously presented): The method according to claim 1, further comprising fusing at least one nucleic acid fragment, that codes for an intracellular targeting signal peptide, into said construct (4), in the same translation reading frame as said third nucleic acid fragment to yield a construct (5), wherein said intracellular targeting signal peptide directs the expression of said single chain antibody to a cellular compartment.

3. (previously presented): The method according to claim 2, wherein said trans-activation peptide in said construct 5 is deleted to yield a construct (6).

4. (previously presented): The method according to claim 2, wherein said transcription associated biomolecule is selected from the group consisting essentially of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of a nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

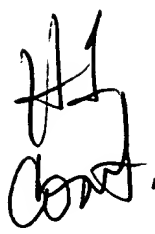
5. (previously presented): The method according to claim 4, wherein said transcription associated biomolecule is selected from the group consisting of Ras, Grb2, phospholipase C γ , phosphatidylinositol 3-kinase, Syp, mitogen activated protein kinase, Jun kinase, androgen receptor, thyroid hormone receptor, glucocorticoid receptor, ATF-1, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM.

6. (previously presented): The method according to claim 3, wherein said transcription associated biomolecule is selected from the group consisting essentially of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of a nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

7. (previously presented): The method according to claim 6, wherein said transcription associated biomolecule is selected from the group consisting of Ras, Grb2,

phospholipase C γ , phosphatidylinositol 3-kinase, Syp, mitogen activated protein kinase, Jun kinase, androgen receptor, thyroid hormone receptor, glucocorticoid receptor, ATF-1, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM.

8. (canceled)

 9. (withdrawn): A single chain monoclonal antibody fusion reagent comprising a single chain antibody fused to a trans-activation peptide, wherein said fusion reagent binds a transcription associated biomolecule within a host cell and is coded by a nucleic acid molecule produced by a method comprising:

(a) cloning a first nucleic acid fragment that codes for a DNA-binding domain peptide of a transcription activator into a first expression vector to yield a construct (1), wherein said DNA-binding domain peptide binds to a DNA regulatory sequence binding site;

(b) fusing a second nucleic acid fragment into said construct (1), in the same translation reading frame as the first nucleic acid fragment, to yield said first expression vector containing a construct (2) that encodes a chimeric DNA-binding domain/transcription associated biomolecule, wherein said second nucleic acid fragment codes for an antigenic portion of said transcription associated biomolecule that is sufficient to generate antibody capable of binding to said transcription associated biomolecule;

(c) providing said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for said DNA-binding domain peptide;

(d) cloning a third DNA fragment that codes for a single chain antibody into a second expression vector to yield a construct (3), wherein said single chain antibody is expressed in a bio-active form that may bind to said antigenic portion;

(e) fusing a fourth nucleic acid fragment that codes for a trans-activation peptide into said construct (3), in the same translation reading frame as the third nucleic acid fragment to yield said expression vector containing a construct (4), encoding a chimeric single chain antibody/trans-activation peptide that may bind to said antigenic portion;

(f) introducing said first and second expression vectors into said host, such that both vectors are expressed;

(g) monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigenic portion within said host cell upon detection of said expression; and

(h) isolating said fusion reagent.

10. (withdrawn): The single chain monoclonal antibody fusion reagent according to claim 9, said method further comprises fusing into said construct (4) at least one nucleic acid fragment that codes for an intracellular targeting signal peptide, in the same translation reading frame as said third nucleic acid fragment, to yield a construct (5), wherein said intracellular targeting signal peptide directs the expression of said single chain antibody to a cellular compartment, whereby said reagent further comprises said intracellular targeting signal peptide fused therewith.

11. (withdrawn): The single chain monoclonal antibody fusion reagent according to claim 9, wherein said trans-activation peptide in said construct 5 is deleted to yield a construct (6).


12. (withdrawn): The single chain monoclonal antibody fusion reagent according to claim 9, wherein said reagent is capable of regulating transcription in said host cell.

13. (withdrawn): The single chain monoclonal antibody fusion reagent according to claim 10, wherein said reagent is capable of regulating transcription in said host cell.

14. (withdrawn): The single chain monoclonal antibody fusion reagent according to claim 11, wherein said reagent is capable of regulating transcription in said host cell.

15. (withdrawn): A therapeutic method for regulating the transcription of a gene associated with a condition or symptom comprises administering an effective amount of a single chain monoclonal antibody fusion reagent that targets a transcription associated biomolecule within a host cell, wherein said fusion reagent is prepared by the steps, comprising:

(a) providing a first expression vector comprised of (i) a first nucleic acid fragment that codes for a DNA binding domain peptide of a transcription activator that binds a DNA regulatory sequence binding site, and (ii) a second nucleic acid fragment that codes for an antigenic portion of said transcription associated biomolecule that is sufficient to generate antibody capable of binding to said transcription associated biomolecule, wherein said first and said second fragments are in the same translation reading frame, whereby said first expression vector encodes a chimeric DNA-binding domain/transcriptional associated biomolecule;

 (b) providing said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for said DNA-binding domain peptide;

(c) providing a second expression vector that comprises (i) a third nucleic acid fragment that codes for a single chain antibody that is expressed in a bio-active form that may bind to an antigen present in said host cell and (ii) a fourth nucleic acid fragment that codes for a trans-activation peptide, wherein said third and fourth fragments are in the same translation reading frame, whereby said second expression vector encodes a chimeric single chain antibody/trans-activation peptide that may bind to said antigenic portion;

(d) introducing said first and second expression vectors into said host cell, such that both vectors are expressed, and

(e) monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigenic portion within said host cells upon detection of said expression.

16. (withdrawn): The therapeutic according to claim 15, said fusion reagent is fused to at least one nucleic acid fragment encoding an intracellular targeting signal peptide.

17. (withdrawn): A method of screening a plurality of compounds for specific binding affinity with a single chain monoclonal antibody fusion reagent according to claim 9 within a host cell, comprising:

(a) providing a plurality of compounds;

(b) contacting said fusion reagent with each of said plurality of compounds for a time sufficient to allow binding under suitable conditions; and

(c) detecting at least some compounds of said plurality that specifically binds to said single chain monoclonal antibody fusion reagent.

18. (withdrawn): A method for diagnosing a physiological disorder manifested by abnormal levels of a transcription associated biomolecule, said method comprising:

(a) contacting a biological sample containing said transcription associated biomolecule with a labeled single chain monoclonal antibody fusion reagent according to claim 9, whereby said reagent binds to said transcription associated biomolecule to form a complex;

(b) separating an unbound labeled reagent from a bound labeled reagent in said complex; and


(c) measuring the amount of said unbound and bound labeled reagents in said complex under identical conditions.

19. (currently amended): A ~~pVP16Zeo-library~~ shuttle expression vector, ~~accorded as ATCC Accession No. 98483,~~ for the construction and screening of a single chain monoclonal antibody fusion reagent, comprising a zeocin selective marker gene to facilitate the isolation and production of said single chain monoclonal antibody fusion reagent in yeast and *E. coli*.

20. (previously presented) A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent within a host cell, comprising:

(a) a first expression vector comprised of (i) a first nucleic acid fragment that codes for a DNA binding domain peptide of a transcription activator that binds a DNA regulatory sequence binding site, and (ii) a second nucleic acid fragment that codes for an antigenic portion of a transcription associated biomolecule, wherein said first and said second fragments are in the same translation reading frame, whereby said first expression vector encodes a chimeric DNA-binding domain/transcriptional associated biomolecule;

(b) a second expression vector that comprises (i) a third nucleic acid fragment from said DNA construct library that codes for a single chain antibody that is expressed in a bio-active form that may bind to said antigenic portion, and (ii) a fourth nucleic acid fragment that codes for a trans-activation peptide, wherein said third and fourth fragments are in the same translation reading frame, whereby said second expression vector encodes a chimeric single chain antibody/trans-activation peptide that may bind to said antigenic portion;

 (c) said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for the DNA-binding domain peptide, for introducing said first and second expression vectors such that both vectors are expressed;

(d) means for monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigen present in said host cell.

21. (previously presented) A kit according to claim 20, further comprises a pVP16Zeo vector, wherein said vector expresses said single chain antibody and is accorded as ATCC Accession No. 98483.

22. (previously presented) A kit according to claim 21, further comprises at least one set of primers.

23. (canceled)

24. (previously presented): A DNA construct according to the method of claim 1.

25. (previously presented): A screening method, comprising:

(a) providing a first expression vector comprised of (i) a first nucleic acid segment that codes for a DNA-binding domain peptide of a transcription activator, wherein said peptide binds a DNA regulatory sequence binding site, and (ii) a second nucleic acid segment that codes for an antigenic portion of said transcription associated biomolecule that is sufficient to generate antibody capable of binding to said transcription associated biomolecule and that is not endogenous to said host cell, wherein said first segment and said second segment are in the same translation reading frame, whereby said first expression vector encodes a chimeric biomolecule;

(b) providing a plurality of host cells containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for said DNA-binding domain peptide;

(c) providing a second expression vector that comprises (i) a third nucleic acid segment that codes for a single chain antibody that may bind said antigenic portion within said host cell and, (ii) a fourth nucleic acid segment that codes for a trans-activation peptide, wherein said third and fourth segments are in the same translation reading frame, whereby said second expression vector encodes a chimeric peptide that binds said antigenic portion;

(d) introducing said first and second expression vectors into at least some host cells of said plurality, such that both vectors are expressed within said host cells; and

(e) monitoring said plurality for expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigenic portion within said host cell upon detection of said expression.

26. (previously presented): The method according to claim 1, wherein said antigenic portion is not endogenous to said host cell.

27. (previously presented): The method according to claim 1, wherein said host cell is a eucaryotic cell.

28. (previously presented): The method according to claim 27, wherein said eucaryotic cell is a yeast or mammalian cell.

29. (previously presented): The method according to claim 1, wherein said detectable gene is a reporter gene or a selectable marker gene.

30. (withdrawn): The reagent according to claim 9, wherein said antigenic portion is not endogenous to said host cell.

31. (withdrawn): The reagent according to claim 9, wherein said host cell is a eucaryotic cell.

32. (withdrawn): The reagent according to claim 31, wherein said eucaryotic cell is a yeast or mammalian cell.

33. (withdrawn): The reagent according to claim 9, wherein said detectable gene is a reporter gene or a selectable marker gene.

34. (withdrawn): The therapeutic method according to claim 15, wherein said antigenic portion is not endogenous to said host cell.

35. (withdrawn): The therapeutic method according to claim 15, wherein said host cell is a eucaryotic cell.

36. (withdrawn): The therapeutic method according to claim 35, wherein said eucaryotic cell is a yeast or mammalian cell.

37. (withdrawn): The therapeutic method according to claim 15, wherein said detectable gene is a reporter gene or a selectable marker gene.

38. (withdrawn): The screening method according to claim 17, wherein said antigenic portion is not endogenous to said host cell.

39. (withdrawn): The screening method according to claim 17, wherein said host cell is a eucaryotic cell.

40. (withdrawn): The screening method according to claim 39, wherein said eucaryotic cell is a yeast or mammalian cell.

41. (withdrawn): The screening method according to claim 17, wherein said detectable gene is a reporter gene or a selectable marker gene.

42. (withdrawn): The diagnostic method according to claim 18, wherein said antigenic portion is not endogenous to said host cell.

43. (withdrawn): The diagnostic method according to claim 18, wherein said host cell is a eucaryotic cell.

44. (withdrawn): The diagnostic method according to claim 43, wherein said eucaryotic cell is a yeast or mammalian cell.

45. (withdrawn): The diagnostic method according to claim 18, wherein said detectable gene is a reporter gene or a selectable marker gene.

46. (previously presented): The kit according to claim 20, wherein said antigenic portion is not endogenous to said host cell.

47. (previously presented): The kit according to claim 20, wherein said host cell is a eucaryotic cell.

48. (previously presented): The kit according to claim 47, wherein said eucaryotic cell is a yeast or mammalian cell.

49. (previously presented): The kit according to claim 20, wherein said detectable gene is a reporter gene or a selectable marker gene.

50. (previously presented): The screening method according to claim 24, wherein said antigenic portion is not endogenous to said host cell.

51. (previously presented): The screening method according to claim 24, wherein said host cell is a eucaryotic cell.

52. (previously presented): The screening method according to claim 51, wherein said eucaryotic cell is a yeast or mammalian cell.

53. (previously presented): The screening method according to claim 24, wherein said detectable gene is a reporter gene or a selectable marker gene.

54. (new): The shuttle expression vector of claim 19, wherein said vector is a pVP16Zeo library expression vector, accorded as ATCC Accession No. 98483.